Nature Conservation 55: 249–268 (2024)
DOI: 10.3897/natureconservation.55.114211

Research Article

Unveiling prey preferences of endangered wild Malayan tiger, *Panthera tigris jacksoni*, in Peninsular Malaysia through scat analysis via *COI* DNA metabarcoding

Millawati Gani[1,2], Frankie Thomas Sitam[2], Zubaidah Kamarudin[3], Siti Suzana Selamat[3], Nik Mohd Zamani Awang[3], Hani Nabilia Muhd-Sahimi[4], Michael Wong[5], Baharim Selat[6], Nur Fatin Khairunnisa Abdullah-Halim[7], Lim Shu Yong[8], Ling Fong Yoke[8], Salmah Yaakop[1], Abd Rahman Mohd-Ridwan[9], Badrul Munir Md-Zain[1]

1 *Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
2 *National Wildlife Forensic Laboratory (NWFL), Ex-Situ Conservation Division, Department of Wildlife and National Parks (PERHILITAN), KM 10 Jalan Cheras, 56100 Kuala Lumpur, Malaysia*
3 *National Wildlife Rescue Centre (NWRC), Department of Wildlife and National Parks (PERHILITAN), 35600 Sungkai, Perak, Malaysia*
4 *Wildlife Conservation Division, Department of Wildlife and National Parks (PERHILITAN), KM 10 Jalan Cheras, 56100 Kuala Lumpur, Malaysia*
5 *PERHILITAN Daerah Mersing, Department of Wildlife and National Parks (PERHILITAN), KM18, Jalan Mersing-Jemaluang, 86800 Mersing, Johor, Malaysia*
6 *Paya Indah Wetlands, Department of Wildlife and National Parks (PERHILITAN), KM 4, Jalan Dengkil-Banting, 43800 Dengkil, Selangor, Malaysia*
7 *Taman Negara Pahang, Kuala Tahan, Department of Wildlife and National Parks (PERHILITAN), 27020 Jerantut, Pahang, Malaysia*
8 *Genomics Platform, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor, Malaysia*
9 *Centre for Pre-University Studies, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia*

Corresponding author: Badrul Munir Md-Zain (abgbadd1966@yahoo.com)

Abstract

Understanding the prey preference of Malayan tiger (*Panthera tigris jacksoni*) in Malaysia is important to guide conservation planning initiatives. The utilisation of DNA metabarcoding provides valuable insights, particularly in the field of carnivora diet research. This technique has been proven to be effective for identifying various species within complex mixtures such as scat materials, where visual identification is challenging. The Cytochrome c oxidase subunit I (*COI*) locus has been selected as it is a widely used as an effective non-invasive approach for diet studies. Hence, given this advance approach, Malayan tiger scats were collected on the basis of existing records of their presence in two types of habitats, namely, protected areas (PA) and human–tiger conflict (HTC) areas. This study aimed to identify prey species in Peninsular Malaysia, based on Malayan tiger scat samples using DNA metabarcoding. Based on the partial mitochondrial *COI* region, DNA metabarcoding led to the taxonomic resolution of prey DNA remnants in scats and the identification of prey species consumed by Malayan tiger, which were predominately small-to-medium-sized prey, including livestock. The dominant DNA prey detected belongs to the family Canidae, followed by Bovidae, Vespertilionidae, Homonidae, Felidae, Phasianidae and Muridae. A significant difference ($p < 0.05$) was observed in alpha and beta diversity using the Shannon index and PERMANOVA with regard to prey richness and evenness in two different habitat groups, namely, PA and HTC. Our finding provides insights into Malayan tiger dietary requirements, which can be used to develop conservation plans and strategies for Malayan tiger, particularly for habitat priorities.

Key words: Diet, faecal, mitochondrial DNA, next-generation sequencing, tiger

OPEN ACCESS

Academic editor: Klaus Henle
Received: 15 October 2023
Accepted: 16 April 2024
Published: 22 May 2024

ZooBank: https://zoobank.org/66AAD5A9-6568-48F4-A5DE-EAF36B28D0E1

Copyright: © Millawati Gani et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Citation: Gani M, Sitam FT, Kamarudin Z, Selamat SS, Awang NMZ, Muhd-Sahimi HN, Wong M, Selat B, Abdullah-Halim NFK, Yong LS, Yoke LF, Yaakop S, Mohd-Ridwan AR, Md-Zain BM (2024) Unveiling prey preferences of endangered wild Malayan tiger, *Panthera tigris jacksoni*, in Peninsular Malaysia through scat analysis via *COI* DNA metabarcoding. Nature Conservation 55: 249–268. https://doi.org/10.3897/natureconservation.55.114211

Introduction

The Malayan tiger (*Panthera tigris jacksoni*) is a prominent apex predator, which has received considerable attention in Malaysia. Peninsular Malaysia constituted approximately 6% of the land areas in Malaysia and is designated as totally protected, which comprises national parks and wildlife reserves managed by the Department of Wildlife and National Parks (PERHILITAN), as well as state parks under the supervision of state governments (Kawanishi et al. 2010). According to Kawanishi et al. (2010), 51% of Malaysia's land cover was identified as a potential tiger habitat, which encompasses 29% confirmed tiger habitat, 9% expected tiger habitat and 13% possible tiger habitat. The potential and expected tiger habitats have decreased in recent times. Malayan tiger requires large prey and vast habitats to survive. However, continuous anthropogenic activities, such as illegal hunting, industrial and agricultural expansion and the establishment of human settlements, have resulted in habitat fragmentation and degradation, which have led to a significant decline in tiger population in Malaysia (Topani 1990; Elagupillay et al. 2001; Kawanishi et al. 2006; Shevade et al. 2017; Ten et al. 2021). At present, less than 200 Malayan tigers are left in the wild habitat (Halim et al. 2019). Other factors that led to the decline of Malayan tiger population include continuous depletion of prey species in the Malayan tiger habitat, which are considered as the current leading threats to tigers. The availability of prey species will determine the behaviour, health, social structure and survival of the Malayan tiger. Tigers can live in a diverse habitat and environment, in which they can consume prey that is of varying sizes and their hunting strategies will change depending on the habitat, prey type and prey size (Karanth 2003).

Predators play a crucial role in shaping the structure of food webs within ecosystems (Ritchie et al. 2012). The selection of prey can alter the composition and function of communities and the predator–prey relationship can affect the structure of habitats, behaviour of populations and their survival rates. Habitat loss and fragmentation also influence the relationship between predator and their prey (Haapakoski et al. 2013). In addition, habitat degradation greatly affects large predators that require a large habitat. The social structure of large predators, such as big cats, depends on the availability of prey biomass (Simcharoen et al. 2014) and the availability of the prey in the wild indicates the resources for their growth, survival and reproduction. The selection of their prey determines their eating habits, which, in turn, determines their life history strategies, including movement, habitat selection, social structure, geographical distribution and reproductive success (Sunquist and Sunquist 1989). However, information regarding real-time prey selection by the Malayan tiger in Peninsular Malaysia is lacking.

Determining the real-time prey selection by Malayan tiger is necessary to recognise the essential conservation needs of this endangered species in the future. Studies on prey selection of predators have been conducted in different ways, such as direct observation and scat analysis through undigested prey items remains in scats, such as hair, fur, bone and nails by using microscopic morphological analysis (Reddy et al. 2004; Ramesh et al. 2009; Kumar 2015; Upadhyaya et al. 2018; Matthews et al. 2020) and a molecular DNA approach (Xiong et al. 2017; Thuo et al. 2019; Hacker et al. 2021; Lu et al. 2021). Direct ob-

servation on the feeding behaviour of Malayan tiger is challenging because of their elusive habitat and limited accessibility in the wild habitat such as feeding areas, time-consuming and low population density. This species is also considered dangerous. Meanwhile, microscopic morphology scat analysis faces misidentification to sympatric analysis (Monterroso et al. 2018) and obtaining an accurate prey list particularly for closely-related prey taxa for species without the hard component that remains in their scat is difficult (Da Silva et al. 2019).

Both approaches have limitation in our study, such as safety during direct observation and the lack of a reference library database for hair, fur and other parts of wildlife. The database remains to be developed by forensic morphology teams at the National Wildlife Forensic Laboratory (PERHILITAN). Apart from scat analysis for undigested prey items to detect a tiger's diet, recent advances on molecular genetics using high-throughput next-generation sequencing (NGS) employing DNA metabarcoding have shown great application potential in determining the current Malayan tiger prey selection. At present, the molecular identification of prey items in scats is used to complement morphological analysis. In recent years, DNA metabarcoding has been widely used to determine various animal taxa diets. A molecular approach to detect prey items from scats is a time- and cost-effective tool (Mumma et al. 2015), which can address the difficulties of detecting prey species consumed by the predator through conventional methods, such as direct observation of feeding behaviour and through microscopic analysis.

The research trend of faecal analysis using DNA metabarcoding in Malaysia has been conducted to various species, such as primate, elephant, bats, birds and insect (Aziz et al. 2017; Chan et al. 2020; Osman et al. 2020, 2022; Fahimee et al. 2021; Abdullah-Fauzi et al. 2022; Mansor et al. 2022; Mohd-Radzi et al. 2022). However, lack of research has been conducted on the wild big cat family using DNA metabarcoding approach, with a specific focus on identifying the gut microbiome composition in the Malayan tiger (Khairulmunir et al. 2023). Considering that alternative diet approaches are difficult to adopt for this species, DNA metabarcoding provides an opportunity to establish the current prey preference of Malayan tigers in Peninsular Malaysia. This study used DNA metabarcoding by targeting the partial *COI* region to determine prey preference of Malayan tiger in their natural habitat. *COI* has been used to identify prey selection by various species, such as snow leopard, primate, giant otter, sea lion, bat, bird and wood mouse in previous studies (Srivathsan et al. 2016; Berry et al. 2017; Bohmann et al. 2018; Rytkönen et al. 2018; Hacker et al. 2021; Quéméré et al. 2021; Tercel et al. 2021; Sato et al. 2022) because of its high resolution of species identification and conserved gene. Therefore, our research aims to detect the prey in Malayan tiger scat using *COI* metabarcoding. Tigers are known to have a diverse habitat preference. In Malaysia, tigers roam areas across a range of forest vegetation types from mountainous to lowland forests. Tigers may be found near forest edges, which are often adjacent to human settlements. These areas can provide a mixture of natural habitat and human-modified landscapes, such as oil palm plantation. Occasionally, tigers may venture into human settlements, leading to conflicts as they may prey on livestock or come into contact with humans. This study categorised habitat preferences into two groups: protected areas (PA) and the forest edge near human settlement areas (human–tiger conflict, HTC), based on the collected

samples. Therefore, this study could provide an insight into their current diet in two different habitats and the information can be used to emphasise the importance of an effective tiger conservation planning strategy.

Methods

Field sampling and sample collections

A total of 33 scat samples were collected from areas known to be inhabited by the Malayan tiger between the years 2021 and 2022. Fig. 1 shows the sampling location in three states of Peninsular Malaysia. Sampling was conducted by trekking and patrolling in the PA and HTC. Field identification of scats during scat sample collections was based on size and shape; however, this strategy is sometimes inconsistent and unreliable as the body size of a carnivore can vary greatly within a species and age group. The difficulty in finding the scat of Malayan tigers in forests may be attributed to the decrease in tiger population in the region or forest fragmentation, which also restricts their movement. During field sampling, all relevant data were collected, including the presence of other animal species and their traces, to determine the potential prey selection of the Malayan tiger in the specific study areas. All potential feline scats found during trekking and searching on the forest floor were collected, regardless of how old they were assumed to be (probably 2 weeks old). Along with the observation of scat size and shape, most scat size was more than 2.5 cm in diameter for tigers and below 2.5 cm for leopard species. In addition, scats were obtained from Malayan tigers that were captured or rescued because of HTC. Scat samples were kept in an icebox during field sampling and preserved at −80 °C prior to DNA extraction.

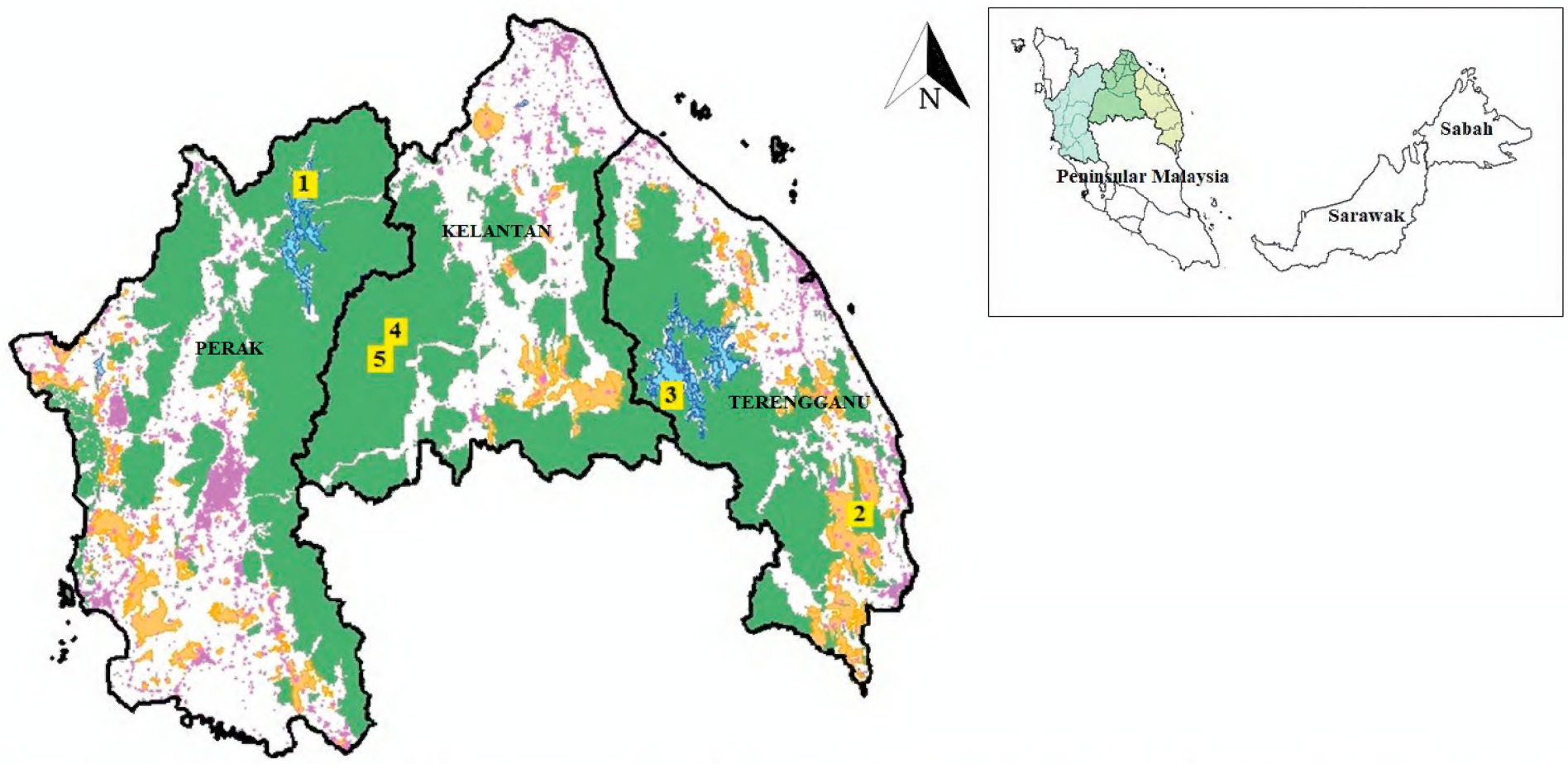

Figure 1. Sampling locations of Malayan tiger scats. (1: Royal Belum State Park, Perak; 2: Felda Kerteh, Kemaman, Terengganu; 3: Taman Negara Terengganu; 4: Pos Bihai Gua Musang, Kelantan; 5: Hutan Simpan Kekal Perias, Kelantan. Map on the left side: green, forest coverage; brown, oil palm plantation; purple, human settlement).

DNA extraction and species identification

All scat samples were extracted using the QIAmp FAST Stool Kit (Qiagen, Germany) and QIAmp DNA Blood and Tissue Kit (Qiagen, Germany). A slight modification was performed during lysis by incorporating DTT to effectively lyse harder components, such as hair and nails present in the scats. In ensuring the absence of cross-contamination, negative controls were included in all DNA extraction procedures and in polymerase chain reactions (PCR). The extracted genomic DNA was visualised using 1% agarose gel electrophoresis and the DNA concentration was determined using Nanodrop ND-1000 (Nanodrop, Wilmington, DE, USA).

Scat samples were amplified using the partial control region (Dloop): MGCR560F (5'-GTGTACCTCTTCGCTCCG-3') and MGCR873R (5'-TGTTGTACGTGGAACCCC-3') for species identification. Of the 33 samples, 13 were successfully amplified and identified as Malayan tiger scats. Given the low concentration of obtained DNA, only 10 Malayan tiger samples were subjected for DNA metabarcoding analysis. Fig. 1 and Table 1 show the scat sample information collected for prey detection data analysis.

PCR and library construction

A total of 10 samples were proceeded with amplicon sequencing as these samples passed the quality control (QC) with the minimum concentration of 10 ng/µl. A two-stage PCR was used to amplify and prepare sequencing libraries. The PCR amplification using the *COI* primer pairs m1COIintF and dgHCO2198 (Geller et al. 2013; Leray et al. 2013). The forward primer, mlCOIintF, was constructed using the Illumina 5' overhang adapter sequence (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG), followed by the forward primer sequence (GGWACWGGWTGAACWGTWTAYCCYCC). Meanwhile, the reverse primer, dgHCO2198R, was constructed using another 5' overhang adapter sequence (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG), followed by the reverse primer sequence (TAAACTTCAGGGTGACCAAARAAYCA). The first PCR step was performed in a 25 µl reaction volume containing 2.5 µl (5 ng/µl) of DNA template, 12.5 µl of KAPA Hifi HotStart ReadyMix (KAPA Biosystem), 0.5 µl (10 µM) of each millilitre

Table 1. Scat sample information of Malayan tigers.

No.	Individuals/Sample ID	Locations tag on map	Habitat type	Habitat type
1.	Syamilla Mek Bihai	4	Kampung Orang Asli	HTC
2.	Sau Bihai	4	Kampung Orang Asli	HTC
3.	PT-BH01	4	Kampung Orang Asli	HTC
4.	Awang Rasau	2	Oil palm plantation	HTC
5.	Atan Kerteh	2	Oil palm plantation	HTC
6.	PSC01	2	Oil palm plantation	HTC
7.	PSC12	3	National Park	PA
8.	PSC13	3	National Park	PA
9.	HSKP-21-13	5	Forest Reserve	PA
10.	SgKejar	1	Forest Reserve	PA

of m1COIintF and dgHCO2198R primers and 9 µl of nuclease-free water. The PCR conditions were as follows: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 45 s and (final extension at?) 72 °C for 10 min. The amplification products of the first PCR were purified using 0.8X AMPure XP beads.

The second round of amplification was performed to incorporate Illumina i5 and i7 adapters and 8-bp barcodes. The PCR mixture was performed in a 10-µl reaction containing 5 µl of KAPA HiFi HotStart Ready Mix, 1 µl of each primer index (i7 and i5) and 3 µl of PCR products from the first PCR. The second PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 8 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 1 min. The libraries were purified using 0.7X AMPure XP beads and quantified using a Qubit 2.0 fluorometer (Life Technologies). The size distribution of the libraries was assessed using TapeStation 2200 (Agilent). All libraries were pooled and loaded on to an Illumina Miseq 2 × 250 bp flow cell at 11 pM. All NGS laboratory works were conducted in Monash University Malaysia Genomics Platform (MUMGP).

Sequences data analysis

Fastq-formatted reads were imported into Geneious Prime® 2022.1.1. The assembly of raw reads of *COI* sequences was run using Geneious Prime® 2022.1.1. Raw sequencing data were trimmed using BBDuk in the Geneious Prime platform to remove any remaining Illumina adapters, bases below the average quality and reads that are less than 150 bp after end-trimming. Then, the trimmed sequences were merged by using the BBMerge tool in the Geneious Prime platform to create a single consensus sequence for each pair. Chimeric sequences were checked and eliminated on the basis of UCHIME (https://drive5.com/uchime/uchime_download.html) and "Gold" database (https://drive5.com/uchime/gold.fa).

Reads were clustered into operational taxonomic units (OTU) using a de novo assembler. Then, the sequences were assigned with a minimum overlap identity of no less than 97%. The taxonomy database was created by blasting the OTUs against a *COI* database from the National Center for Biotechnology Information (NCBI) nucleotide database with some entrez filters targeting on Eukaryote sequences and excluding environmental, uncultured and unclassified sequences. After creating a sequence classifier database from BLAST hits, the full amplicon dataset was classified using the Sequence Classifier in Geneious Prime 2022.1.1. Then, the filtered reads were analysed using R version 2022.07.2. All analyses, including the determination of relative read abundance (RRA), as well as alpha and beta diversity analyses, were performed to evaluate within and between sample habitat types by using the phyloseq (McMurdie and Holmes 2013) and ggplot2 (Wickham 2016) packages in Rstudio. Alpha diversity indices were estimated to indicate the species richness, abundance and evenness of each sample. The alpha diversity using Chao1 and Shannon–Weaver indices was calculated to describe the population diversity in scat samples (Kim et al. 2017). Beta diversity was calculated using weighted unifrac and pairwise Bray–Curtis index to construct Principal Coordinate Analysis (PCoA), which represents the differences between prey selection and habitat groups in HTC and PA. PERMANOVA with 999 permutations was also used to estimate and assess significant differences in diet amongst habitat groups.

Data accessibility

Raw sequence reads have been archived on the NCBI Sequence Read Archive (SRA) with project number PRJNA954969, Biosamples submission numbers SAMN34161777, SAMN34161791, SAMN34161871, SAMN34162214, SAMN34162338, SAMN34162396, SAMN34163286, SAMN34163462, SAMN34163485 and SAMN34163613.

Results

Scat species identification and data filtering

Of the 33 felid scats collected during field sampling, only 13 were confirmed to be Malayan tigers. However, only 10 gDNA products of Malayan tiger scats were proceeded to NGS because of low DNA concentration. DNA metabarcoding analysis was run in Illumina Miseq platform and a total of 2,132,729 raw reads were generated after filtering. Then, low-quality sequences, primer adapter and chimeric reads were removed and 440,272 reads remained. The remaining metabarcoding *COI* data were blasted against the NCBI nucleotide database. The rarefaction curve with 13,885 reads (Fig. 2) showed that the samples reached an asymptote and sampling depth was sufficient to estimate the diversity. Table 2 shows the read count summary generated during filtering and rarefaction.

Malayan tiger dietary profiling

A total of 10 Malayan tiger scat samples indicated the presence of 416 OTUs and yielded 17 phyla in the kingdom of eukaryotes (Fig. 3A). This study aims to identify the potential prey species predated by Malayan tiger are in phylum Chordata only. Therefore, 21 OTUs were generated from the 97% OTU cluster in phylum Chordata. Three classes of prey DNA were identified in 10 Malayan tiger scats, comprising seven orders and eight families and only 12 genera were identified.

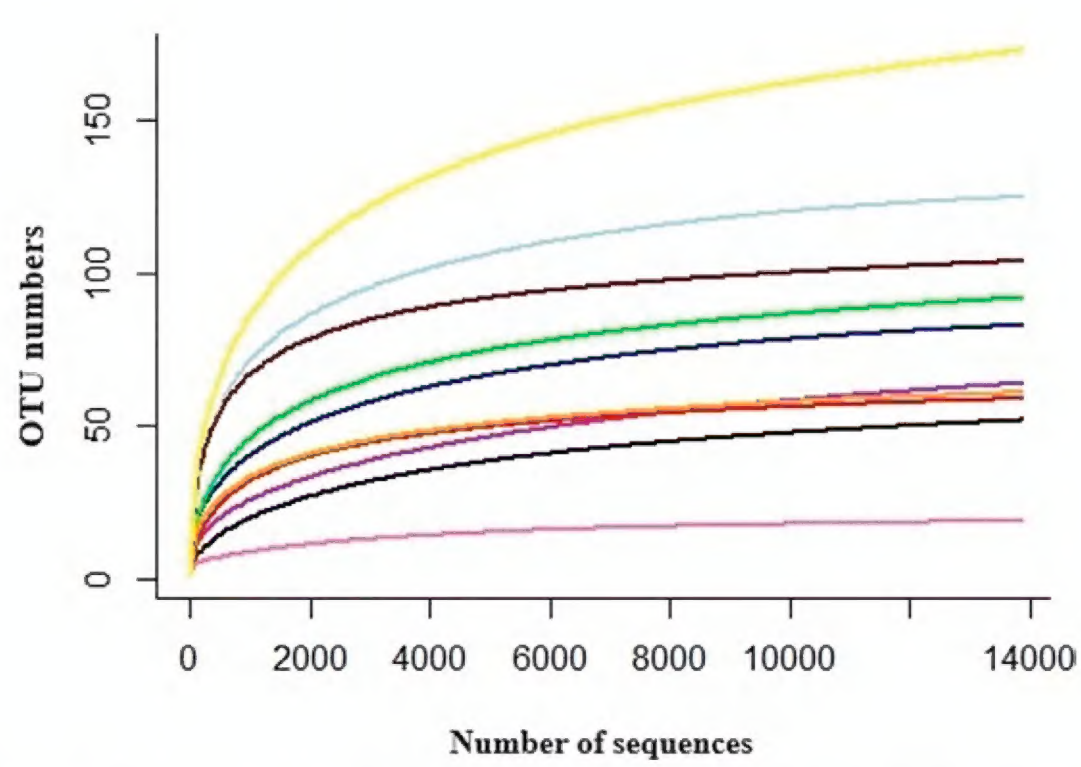

Figure 2. Rarefaction curve based on 10 samples of wild Malayan tiger scats.

Table 2. Read count summary of 10 Malayan tigers.

Sample ID	Habitat type	OTU numbers	Raw sequence reads	Non-chimeric reads
Syamilla Mek Bihai	HTC	81	187,410	80,100
Sau Bihai	HTC	73	146,716	43,777
PT-BH01	HTC	116	159,839	59,362
Awang Rasau	HTC	71	176,472	55,069
Atan Kerteh	HTC	110	203,531	26,232
PSC01	HTC	26	247,962	32,600
PSC12	PA	73	249,590	35,862
PSC13	PA	131	246,986	13,885
HSKP-21-13	PA	120	182,621	51,093
SgKejar	PA	219	331,602	42,382

Despite unknown and unidentified Chordata sequences, Malayan tiger scats were dominated by the class Mammalia (99.96%), Aves (0.01%) and Reptile (0.03%). These percentages were derived from the 3.56% representation of the Chordata phylum, which was generated from all COI sequences obtained. The three classes identified consist of seven orders which are Artiodactyla (22.52%), Carnivora (71.38%), Chiroptera (5.67%), Galliformes (0.04%), Primates (0.34%), Rodentia (0.05%) and Squamata (0.01%).

Considering the habitat type, Malayan tigers at PA preyed most on the family Canidae (66.21%), followed by Vespertilionidae (5.66%), Bovidae (1.75%) and Rhinolophidae (0.01%). Meanwhile, the dominant prey DNA was identified in Malayan tiger scats that are living in areas categorised as HTC mostly from the family Bovidae (20.74%), Homonidae (0.34%), Felidae (Felinae, 0.06%), Muridae (0.044%) and Phasianidae (0.038%, Fig. 4A). Fig. 5 shows the summary of the overall RRA of prey DNA in 10 tiger scats within the two habitat groups based on relative abundance at genus level. The HTC and PA groups showed different patterns of prey selection by Malayan tigers. In the HTC group, the primary prey

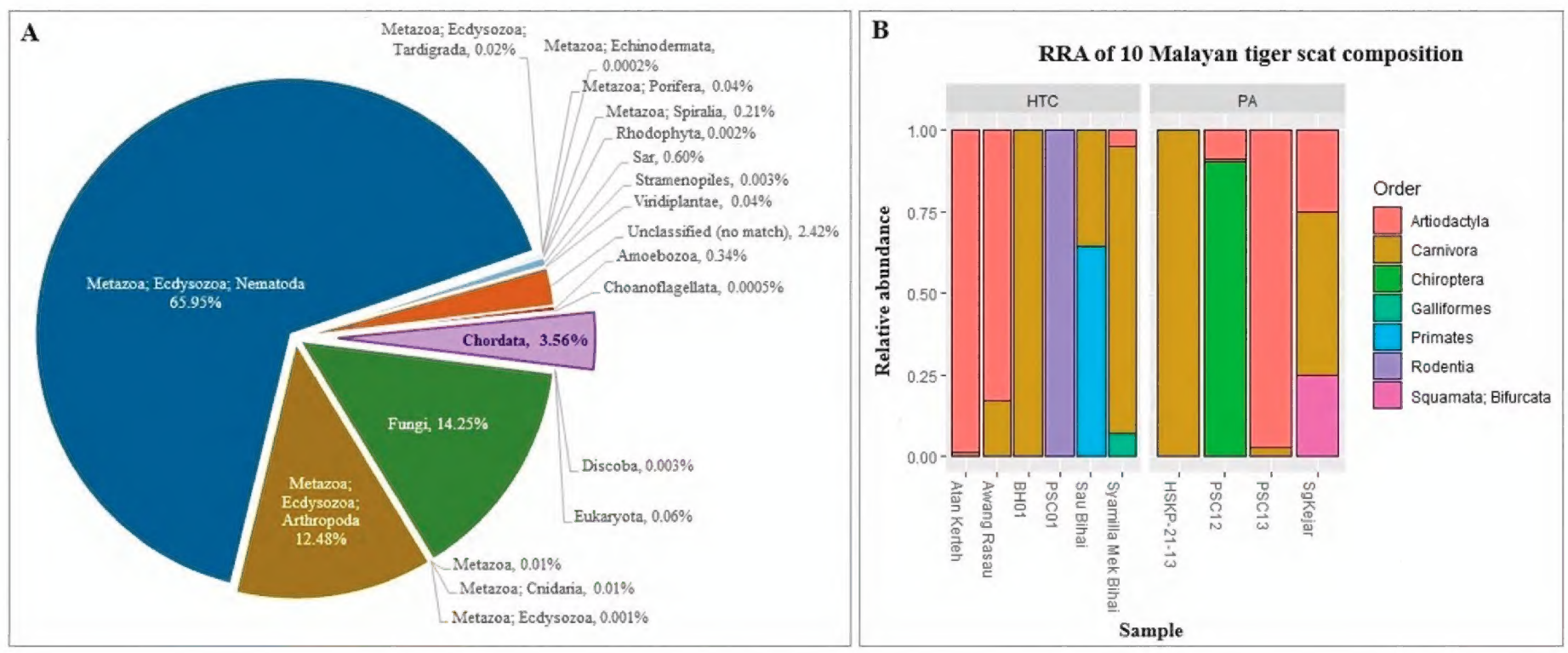

Figure 3. Scat composition of 10 Malayan tigers **A** relative read abundance (RRA) of scat composition at phylum level, based on 416 OTUs and **B** RRA of scat composition from two habitat groups of Malayan tigers at order level, based on 3.56% Chordata phyla identified.

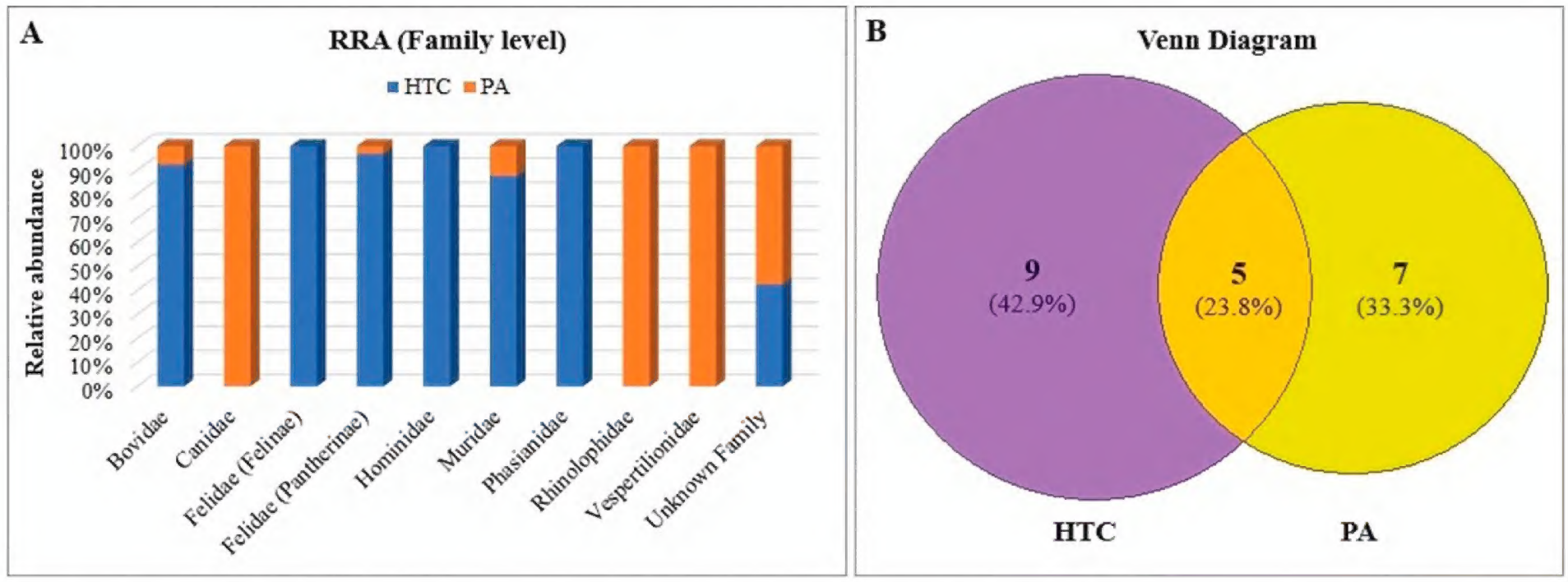

Figure 4. Prey taxa composition in scat samples of Malayan tigers, based on 21 OTUs **A** relative read abundance (RRA) of prey taxa at family level in Malayan tiger scats and **B** venn diagram from two habitat groups of Malayan tigers.

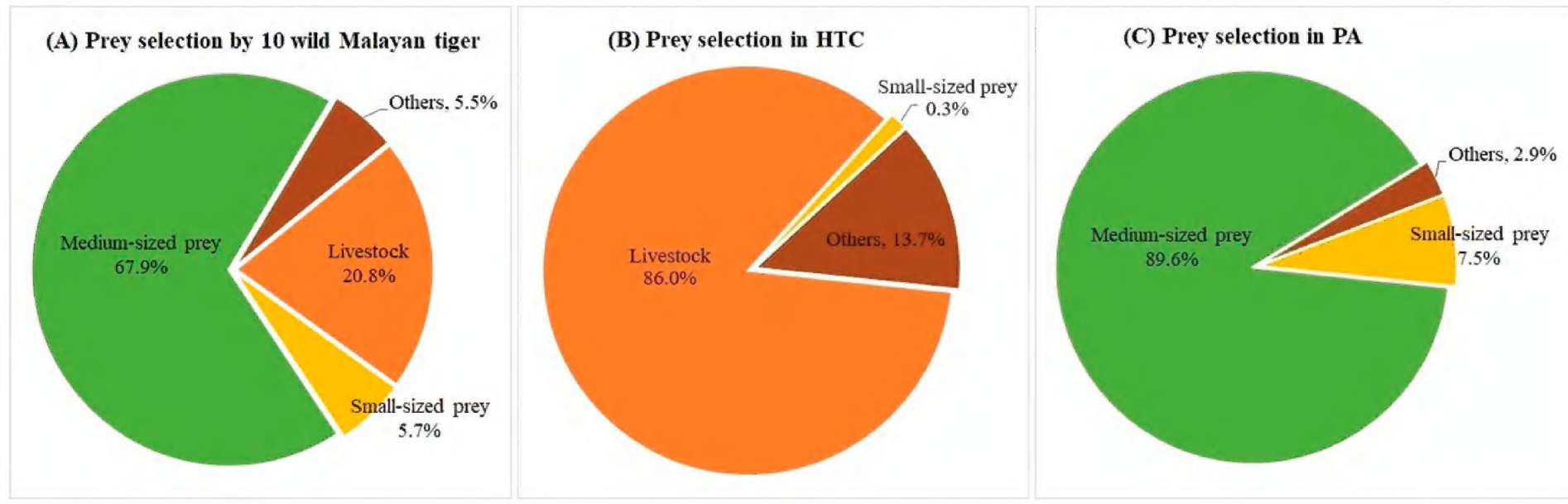

Figure 5. Summary of prey species identified in **A** all Malayan tiger scats **B** human–tiger conflict areas (HTC) and **C** protected forest areas (PA).

selection of tigers primarily consists of livestock (86.0%), small prey (0.3%), followed by other prey species (14%). The livestock consist of genus *Bos*, *Bubalus*, *Capra* and *Gallus*. Small-size prey identified include genus *Mus* and *Felis*. Meanwhile, for the other group, this consists of genus *Homo*, Malayan tiger DNA itself and unknown genus. The PA group showed the absence of livestock species and preyed on medium (89.6%) and small (7.5%) prey. The medium prey consists of genus *Canis* and *Capricornis*; meanwhile, small prey consists of genus *Kerivoula*, *Rhinolophus* and *Mus*. The Venn diagram shows (Fig. 4B) the 97% OTU cluster overlap within the HTC and PA groups. The size of the circle represents the relative OTU richness of each habitat type, PA (7 OTUs) and HTC (9 OTUs). A total of five shared OTUs between PA and HTC indicate that preys were commonly consumed by Malayan tigers in both habitat types.

Alpha and beta diversity

Species richness in each sample was assessed for alpha diversity analysis using the Chao1 and Shannon indices. The Shannon index showed a significant difference in prey selection by Malayan tigers at two different habitat groups

($p < 0.05$), whereas the Chao1 index showed no significant difference ($p > 0.05$). The high value of the Shannon index indicates the high diversity in richness and evenness. Meanwhile, the Chao1 index is a non-parametric method for estimating the number of species in a community. Table 3 shows the score values of Chao1 and Shannon indices and Fig. 6A shows the Chao1 and Shannon indices within the HTC and PA groups. The Chao1 and Shannon indices showed higher prey diversity in the PA group.

Beta diversity was displayed using a PCoA plot of *COI*-rarefied RRA data of prey DNA in Malayan tiger scats in different habitat types (Fig. 6B). A Bray–Curtis distance was calculated on the basis of the pairwise taxonomic profile of 10 scat samples and used to generate PCoA coordinates of each sample. The distance linking two samples is shorter, indicating higher similarity between these samples. The first (PCoA1) and second (PCoA2) axes explained the total variation of 13.8% and 12.2%, respectively. Samples collected from the two habitats

Table 3. Values of Chao1 and Shannon indices of 10 samples.

Samples ID	Chao1 index	Shannon index
Syamilla Mek Bihai	88.5	1.47674
Sau Bihai	106.0	0.67834
PT-BH01	129.6	2.38472
Atan Kerteh	141.6	2.37876
Awang Rasau	76.6	0.97147
PSC01	32.0	0.78397
PSC12	82.2	2.03072
PSC13	143.4	2.88448
HSKP-21-13	130.1	3.13198
SGKejar	235.1	3.38908

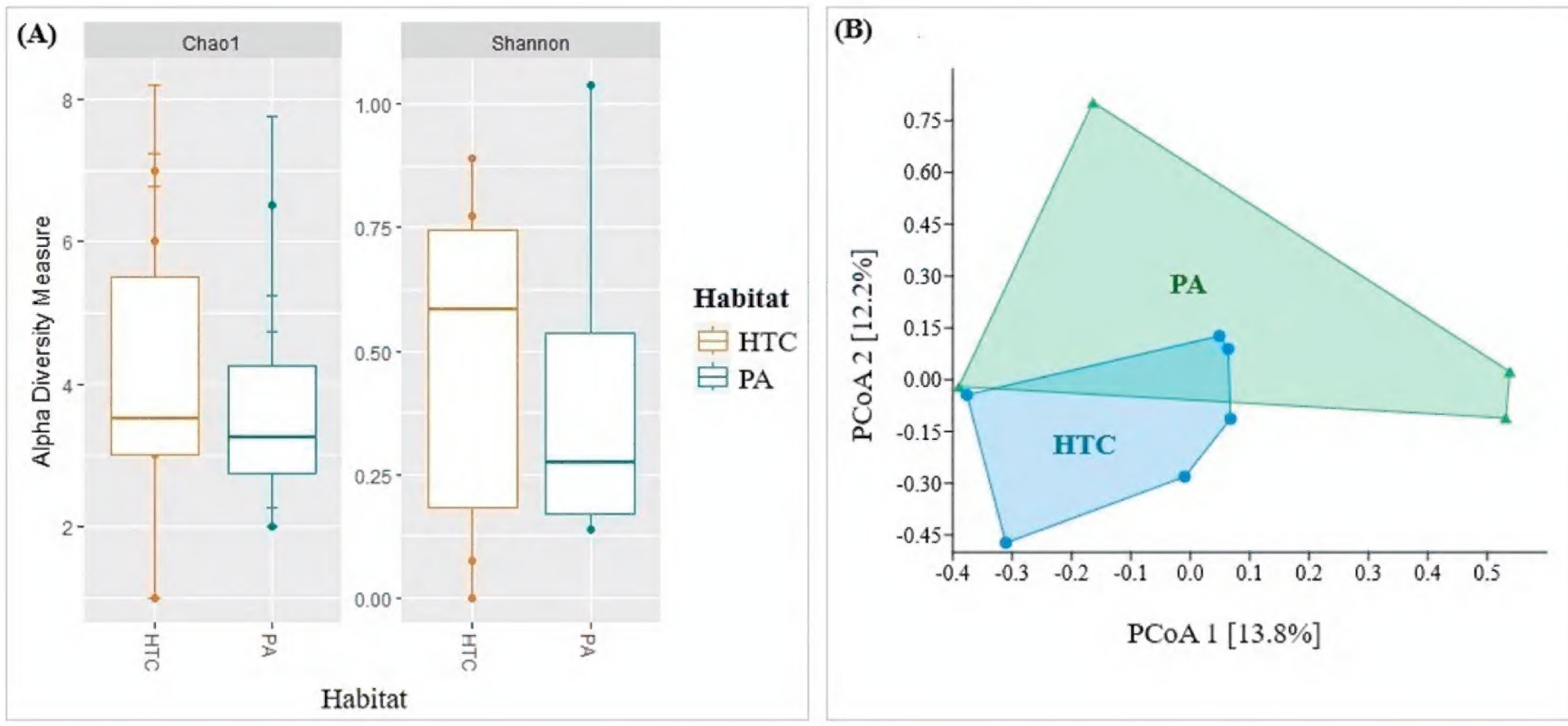

Figure 6. Diversity of prey taxa in Malayan tiger scat samples **A** alpha diversity, based on Chao1 and Shannon indices. **B** beta diversity displayed in the PCoA plot, based on the Bray–Curtis distance.

were illustrated by different colours. The PCoA plot data indicated there is a significant difference between HTC and PA. This result was supported by PERMANOVA analysis, which showed significant difference between the PA and HTC groups (Pr(> F) = 0.326; R^2 = 0.117; F = 1.0624, *p*-value = 0.026, < 0.05).

Discussion

Malayan tiger is an apex predator that plays a vital role in the ecosystem in Malaysia. The abundance and occurrence of prey species in tiger's habitat are associated with the predator–prey interaction, including survival, behaviour and their movement areas. In this study, scat sample collection was conducted in the wild habitat of Malayan tiger to understand the selection of prey by Malayan tiger in Peninsular Malaysia. Fig. 7 illustrates 10 scat samples confirmed to be from Malayan tiger, identified through molecular detection of the mitochondrial DNA (mtDNA) D-loop region. The taxonomic classification analysis in this study indicates that only 3.56% of phylum Chordata *COI* sequences were generated in 10 scat samples. The most sequences generated in this study are largely identified *COI* sequences of the fungi, Choanoflagellata, Amoebozoa, Discoba, Sar, Viridiplantae other metazoas and unknown eukaryotes (94.02%). Another 2.42% of *COI* sequences could not be assigned to any taxonomic group (unknown/unclassified sequences). This is probably due to sample quality and primer sensitivity to detect DNA prey items in scats of Malayan tiger.

This study found that the scat composition of the Malayan tiger in the selected areas of Peninsular Malaysia was dominated by medium prey (67.9%), livestock (20.8%) and small prey (5.7%, Fig. 5A). Based on our findings, Malayan tiger prey varies in HTC compared with that in PA areas. Malayan tiger prey selection in PA areas comprises dogs, bats, serows, rodents and squamates. Meanwhile, the dominant prey DNA identified in the HTC areas comprises prey taxa from livestock species (cow, buffalo, goat, chicken), rodent, human and cats. Alpha (Shannon index) and beta diversity (PERMANOVA) analysis showed significant difference ($p < 0.05$) between the PA and HTC groups. This finding might be due to the habitat characteristics, which influence the selection of prey availability in each habitat group. Therefore, the significant differences between the PA and HTC groups are also because of the differences in prey composition found in tiger scats in the HTC group, which are livestock, as well as the detection of human DNA in the HTC group. As shown in Fig. 5, livestock and human DNA were detected in the HTC group; meanwhile, these prey taxa were not found in the PA group. In addition, DNA from humans was discovered in Malayan tiger scat samples collected from the HTC group. This finding is attributed to two scat samples that originated from tigers involved in previous human attacks. The changes in behaviour and habitat structure to the food requirement bring Malayan tigers into conflict with humans and livestock. Department of Wildlife and National Parks (2008) reported that Malayan tigers also hunt livestock that are near to tiger habitats. Recently, Malayan tigers were found closer to human settlement areas and oil palm plantations because of the availability of livestock where they can easily hunt and readily prey on the livestock. The occurrence of tigers hunting livestock can be ascribed to the migration of Malayan tigers towards areas where livestock is present, as well as with their inability to fulfil their essential dietary requirements within their native

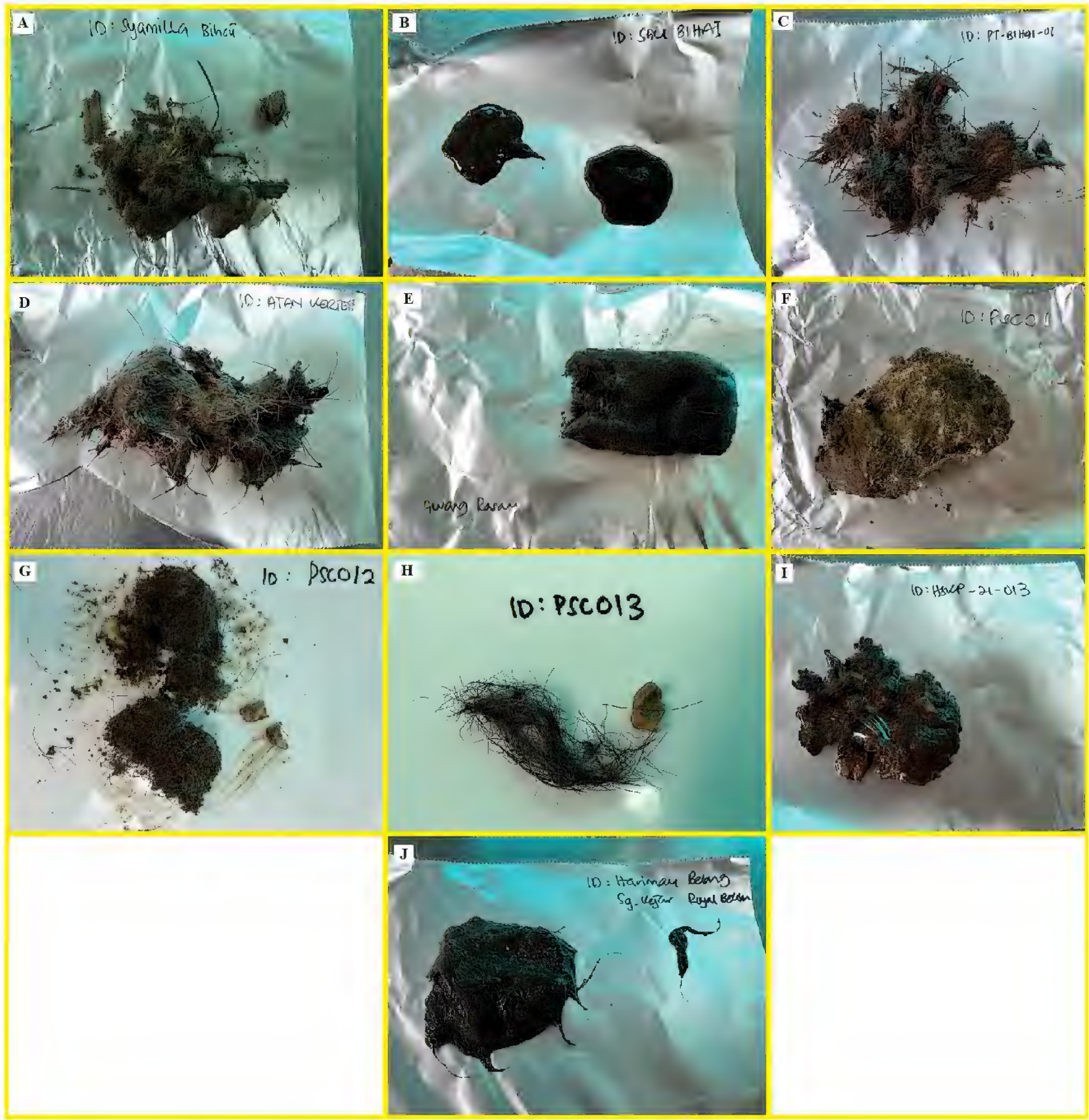

Figure 7. Collected Malayan tiger faecal samples **A** Syamilla Mek Bihai **B** Sau Bihai **C** PT-BH01 **D** Atan Kerteh **E** Awang Rasau **F** PSC01 **G** PSC12 **H** PSC13 **I** HSKP-21-13 **J** SgKejar.

habitats (Kumar 2015). Consequently, tigers shift from forested areas to buffer zones as they are compelled to do so when they find more livestock accessible and easy to prey upon (Kolipaka et al. 2017).

Scat composition analysis of Malayan tigers inhabiting PA areas detected the presence of dogs DNA in HSKP-21-13 sample, serow's DNA from PSC12 and PSC13; meanwhile bats, rodent and squamate DNA have been found in the scat sample named PSC12. Notably, the *COI* taxonomic classification analysis in this study did not detect any common prey by Malayan tiger as reported in previous studies (Lynam et al. 2007; Kawanishi et al. 2010) such as wild pig, barking deer and sambar deer. There is no large prey species found in Malayan

tiger scat samples in the PA group. In the forest area, probably large prey was scarce; thus, hunting large prey is also scarce. Therefore, when the main prey was scarce, tigers will alter their diet. This factor could explain why tigers prefer to non-selectively prey on small-to-medium-sized species. We hypothesised that Malayan tigers in Peninsular Malaysia have a wider range of available prey sizes to meet their dietary requirements. Small sized prey consumed by tigers were rarely reported by previous studies. However, based on a study conducted by Schaller (1967), Royal Bengal Tigers also engage in predation on smaller species such as civet cats, water monitors, birds, fish and frogs, apart from their high consumption of spotted deer, wild boar, rhesus monkeys and water monitors. This finding is coherent with our study, demonstrating that tigers across different subspecies can prey on smaller animals, irrespective of the analysis method used. Bats have been identified in a scat sample labelled as PSC12. The probable explanation for detecting these bat species in the analysis of Malayan tiger scats is the significant bat population present reported in the PSC12 location (Pounsin et al. 2018; Azmir et al. 2022). The area comprises of hill dipterocarp forests and nearby karst caves. The vicinity of karst caves to the collected scat samples suggests the possibility of Malayan tigers preying on bats in their roosting areas. According to National Tiger Survey (NTS) programme which was coordinated by PERHILITAN, the Malayan tiger has been recorded roaming around the cave areas through the camera trap (Halim et al. 2019). Therefore, tiger prey preference may vary depending on locations. Another possibility is that the Malayan tiger indirectly ingested the small-sized animal while predating on another prey.

In addition to the above, during field sampling, wild boar is found to be present in PA and HTC areas; however, no wild boar DNA were detected in tiger scats in our analysis. Kawanishi et al. (2003) assumed that the abundance of wild boar and omnipresence in almost all vegetation, including oil palm plantation, can be the main option of prey by Malayan tigers, although some of the deer populations were depleted. However, tigers preferred to prey on livestock and small-to-medium-sized species to meet their food dietary requirement in PA and HTC areas. The reason why tigers prefer livestock, small and other medium species rather than wild boar in this study remains unknown. Thus, investigating the assertion and the degree to which the tiger's diet is augmented by livestock is important to understand the real productivity of the tiger's habitat beyond what is naturally available (Kawanishi et al. 2003). As mentioned before, tiger prey may vary depending on locations; however, in general, wild boar and sambar deer are the foremost kinds of herbivorous mammals that have been linked to tigers as prey (Reddy et al. 2004; Ramesh et al. 2009; Mukherjee and Sarkar 2013; Kumar 2015; Upadhyaya et al. 2018).

Based on the findings of this study, the prey DNA in Malayan tiger scats may be influenced by other factors such as the scat sample quality, predation time, and selection of gene used. The detection of DNA prey depends on several factors, particularly the time when Malayan tigers consumed their prey species. Some DNA were degraded because of long exposure to the environment and the freshness of the scat sample affected the detection and identification of prey species (Upadhyaya et al. 2018). Thuo et al. (2019) conducted a study on the influence of various factors on prey DNA detection, such as the time of prey consumption (feeding day), scat age, prey species and meal size. The study

revealed that prey DNA detection was weaker on day 0, the day the prey was consumed by cheetahs. However, DNA was high on day 1 and then it declined on the following day. Moreover, chicken, deer and horse were easily detected on the day of consumption compared with quail and rabbit, based on the prey species. In addition, the assessment of prey species identification in scats was affected by prey size. According to Ackerman et al. (1984), small prey species have a high amount of hair per unit body weight, which results in undigested hair found in their scat. The presence of a large amount of hair in the scat could result in an exaggerated observation of the occurrence of smaller prey species in the diet of the carnivore. This study revealed a lower frequency of prey species occurrence in each scat. Several factors could influence this frequency, including: 1) samples size, 2) sample quality or 3) the timing of predation (whether the prey species had been recently hunted or had been hunted for an extended period). Therefore, this study recommends future studies to increase sample number by conducting a longer duration of field sampling and covering a wider area of study locations.

Another factor influencing the result is the selection of gene or primers for prey DNA detection in scat samples. In this study, taxonomic classification analysis indicates that 2.42% of *COI* sequences generated could not be assigned to any taxonomic group (unknown/unclassified sequences). The partial *COI* gene (mlCOIintF and dgHCO2198R) can identify only 3.56% of the Chordata taxa in all samples and another 94.02% of the sequences generated largely identified other eukaryotes, such as fungi, amoebozoa, viridiplantae and another metazoan other than Chordata. The partial region of *COI* used in this study is an insufficient taxonomic coverage of *COI* barcoding primers. Hacker et al. (2021) conducted a study utilising the *COI* region on *Panthera uncia* to analyse MT-*COI* and identify the prey items, particularly Caprinae, which is present in snow leopard scats. However, they found that only goat and sheep DNA were successfully amplified, whereas the DNA of other ungulate families or mammalian orders was not detected. DNA metabarcoding has its own shortfalls and it depends on the accuracy and coverage of the DNA reference database (Nielsen et al. 2018; Traugott et al. 2020). The *COI* primer might be used in identifying prey species from invertebrates, but not much for vertebrate prey species. Moreover, using a single set of primers focusing on specific prey groups may produce biased results (Da Silva et al. 2019). According to Quéméré et al. (2021), the use of multiple primers can produce more comprehensive results. Utilising multiple markers enhances the precision in identifying individual taxa and contributes to the overall precision of dietary profiles (Ando et al. 2020). Therefore, this study suggests that future studies should consider incorporating multiple loci or other alternative loci to investigate the elusive prey selection by the Malayan tiger in Peninsular Malaysia.

Conclusions

This study was the first to investigate and describe the prey selection of Malayan tigers by DNA metabarcoding of scats. Despite the relatively small number of scats analysed in this study, the result demonstrated the overview of the current situation of prey selection by Malayan tigers in Peninsular Malaysia. In HTC and PA areas, Malayan tigers mostly consumed livestock and medi-

um-to-small-sized prey species. Human expansion might be the major cause of the alteration of prey selection to livestock and medium-to-small-sized prey species. Prey selection by Malayan tigers is affected by human-induced alterations to the environment. Forest fragmentation causes the roaming areas of Malayan tiger to move towards the human areas, particularly oil palm plantation areas and human settlements. Usually, livestock owned by local people are often placed within their oil palm plantations area and most of the oil palm plantations in Peninsular Malaysia were near to the forest making the area a HTC area. When livestock is available, tigers will readily prey on them. Based on our results, future studies on tiger diet should consider using multi-locus DNA metabarcoding and conducting field sampling for a longer duration and should cover a wide area to understand the spatiotemporal variation in tiger diet. This approach will provide other opportunities to study their preference in the wild. Although this study cannot be used to quantify true abundance or proportion of prey species, it provides an important first step towards identifying prey taxa and spatial–temporal patterns in Malayan tiger diets. Less attention has been paid to medium and small prey than to large ones, leading to a shortfall in knowledge regarding their ecological roles. This limitation should be considered to help in planning strategies of conservation effort to Malayan tigers in Peninsular Malaysia. The data obtained in this study will improve dietary insight, which can be used to develop conservation plans and strategies for Malayan tigers, particularly for habitat priorities, protection and restoration in specific areas.

Acknowledgements

We wish to thank the Director General (YBhg. Dato' Abdul Kadir bin Abu Hashim) and Director of the Ex-situ Conservation Division of PERHILITAN Peninsular Malaysia (Dr. Pazil Abdul Patah) for the support and permission to conduct this study (Permit No.: P06/10/2020). We are also thankful to the Universiti Kebangsaan Malaysia for the ethical permit approval in our research protocol (UKMAEC approval number: FST/2021/BADRUL MUNIR/22-SEPT./1198-OCT.-2021-OCT.-2023-NAR-CAT2). We would especially like to thank the MyTAG teams, staff of Ex-Situ Conservation Division, National Wildlife Rescue Centre (NWRC), Carnivore Unit Wildlife Conservation Division, PERHILITAN Terengganu, PERHILITAN Kelantan, PERHILITAN Perak, Royal Belum State Park, for their assistance in handling tigers during field sampling at HTC areas. Acknowledged also are the teams from SPARTA PERHILITAN, Primate Unit Wildlife Conservation Division PERHILITAN, Inventory Unit Wildlife Conservation Division PERHILITAN and Panthera Malaysia who helped with sample collections.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

Wildlife Permit No.: P06/10/2020 -Ethical Permit: UKMAEC approval number: FST/2021/BADRUL MUNIR/22-SEPT./1198-OCT.-2021-OCT.-2023-NAR-CAT2).

Funding

The authors acknowledge the Fundamental Research Grant Scheme (FRGS), FRGS/1/2020/WAB11/UKM/01/1 funded by the Ministry of Higher Education (MOHE), Malaysia and the part for this research is supported by the Government of Malaysia under the 12th Malaysia Plan Project: Strengthening Wildlife Forensics, Ex-Situ Conservation and Biobanking-Phase 2 (Project Code: P23071000810008) and Geran Universiti Penyelidikan (GUP), grant number GUP-2022-043 Universiti Kebangsaan Malaysia.

Author contributions

MG, FTS, ZK, SSS, NMZA, HNMS, MW, BS and NFKAH conducted field sampling and collected samples. MG conducted DNA extraction and laboratory works. LSY and LFY performed library construction and sequencing. MG analysed all the data and collected information of the samples. MG drafted and edited the manuscript. ARMR, SY and BMMZ critically revised the intellectual content. All authors read and approved the final version of the manuscript.

Author ORCIDs

Millawati Gani https://orcid.org/0000-0002-9508-7481
Lim Shu Yong https://orcid.org/0000-0003-2232-8261
Ling Fong Yoke https://orcid.org/0000-0002-1696-4969
Salmah Yaakop https://orcid.org/0000-0002-2998-8716
Abd Rahman Mohd-Ridwan https://orcid.org/0000-0003-0052-4149
Badrul Munir Md-Zain https://orcid.org/0000-0003-4037-8115

Data availability

All of the data that support the findings of this study are available in the main text.

References

Abdullah-Fauzi NAF, Karuppannan KV, Mohd-Radzi NHS, Gani M, Mohd-Ridwan AR, Othman N, Haris H, Sariyati NH, Aifat NR, Abdul-Latiff MAB, Abdul-Razak MFA, Md-Zain BM (2022) Determining the dietary preferences of wild Asian elephants (*Elephas maximus*) in Taman Negara National Park, Malaysia based on sex and age using trnL DNA metabarcoding analysis. Zoological Studies 61: 60. https://doi.org/10.6620/ZS.2022.61-60

Ackerman BB, Lindzey FG, Flemker TP (1984) Cougar food habits in southern Utah. The Journal of Wildlife Management 48(1): 147–155. https://doi.org/10.2307/3808462

Ando H, Mukai H, Komura T, Dewi T, Ando M, Isagi Y (2020) Methodological trends and perspectives of animal dietary studies by noninvasive fecal DNA metabarcoding. Environmental DNA 2(4): 391–406. https://doi.org/10.1002/edn3.117

Aziz SA, Clements GR, Peng LY, Campos-Arceiz A, McConkey KR, Forget P, Gan HM (2017) Elucidating the diet of the island flying fox (*Pteropus hypomelanus*) in Peninsular Malaysia through illumina next-generation sequencing. PeerJ 5: e3176. https://doi.org/10.7717/peerj.3176

Azmir K, Aman M, Rahmat M, Yahya N, Samsuddin N, Tarmizi N, Jusof N, Kamaruddin N, Hassan Basri H, Aznan S, Marni W, Zahidin M, Assyuhada MG, Edinur H, Abdullah MT (2022) Assessment of Tanjung Bewah karst at Tasik Kenyir and construction of a framework for high value biodiversity area. Malayan Nature Journal 73: 459–468.

Berry TE, Osterrieder SK, Murray DC, Coghlan ML, Richardson AJ, Grealy AK, Stat M, Bejder L, Bunce M (2017) DNA metabarcoding for diet analysis and biodiversity: A

case study using the endangered Australian sea lion (*Neophoca cinerea*). Ecology and Evolution 2017(7): 5435–5453. https://doi.org/10.1002/ece3.3123

Bohmann K, Gopalakrishnan S, Nielsen M, Santos LD, Nielsen B, Jones G, Streicker DG, Gilbert TP (2018) Using DNA metabarcoding for simultaneous inference of common vampire bat diet and population structure. Molecular Ecology Resources 18(5): 1050–1063. https://doi.org/10.1111/1755-0998.12891

Chan KS, Tan J, Goh WL (2020) Taxonomic assignment of dietary arthropods in Malaysian swiftlets (*Aerodramus sp.*) based on DNA metabarcoding. Journal of Wildlife and National Parks 35: 73–91.

Da Silva LP, Mata VA, Lopes PB, Pereira P, Jarman SN, Lopes RJ, Beja P (2019) Advancing the integration of multi-marker metabarcoding data in dietary analysis of trophic generalists. Molecular Ecology Resources 19(6): 1420–1432. https://doi.org/10.1111/1755-0998.13060

Department of Wildlife and National Parks (2008) National Tiger Action Plan for Malaysia. DWNP Kuala Lumpur, Malaysia.

Elagupillay ST, Laidlaw R, Wan-Shahruddin WN (2001) Tiger conservation and the Department of Wildlife and National Parks (DWNP) Peninsular Malaysia. Journal of Wildlife and Parks 19: 89–97.

Fahimee J, Badrulisham AS, Zulidzham MS, Reward NF, Muzammil N, Jajuli R, Md-Zain BM, Yaakop S (2021) Metabarcoding in Diet Assessment of *Heterotrigona itama* based on trnL Marker towards Domestication Program. Insects 12(3): 205. https://doi.org/10.3390/insects12030205

Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources 13(5): 851–861. https://doi.org/10.1111/1755-0998.12138

Haapakoski M, Sundell J, Ylönen H (2013) Mammalian predator–prey interaction in a fragmented landscape: Weasels and voles. Oecologia 173(4): 1227–1235. https://doi.org/10.1007/s00442-013-2691-y

Hacker C, Jevit M, Hussain S, Muhammad G, Munkhtsog B, Munkhtsog B, Zhang Y, Li D, Liu Y, Farrington JD, Balbakova F, Alamanov A, Kurmanaliev O, Buyanaa C, Bayandonoi G, Ochirjav M, Liang X, Bian X, Weckworth B, Jackson R, Janecka JE (2021) Regional comparison Snow Leopard (*Panthera uncia*) diet using DNA metabarcoding. Biodiversity and Conservation 30(3): 797–817. https://doi.org/10.1007/s10531-021-02118-6

Halim HRA, Mustapha N, Ibrahim NL (2019) The Story of Malayan Tiger. Department of Wildlife and National Parks, Cheras, Malaysia.

Karanth KU (2003) Tiger ecology and conservation in the Indian subcontinent. Journal of the Bombay Natural History Society 100: 169–189.

Kawanishi K, Yatim SH, Hashim AKA, Topani R (2003) Distribution and potential population size of the tiger in Peninsular Malaysia. Journal of Wildlife and Parks (Malaysia) 21: 29–50.

Kawanishi K, Sunquist ME, Jasmi A (2006) Effects of human activities on the tiger-prey community in Taman Negara National Park, Peninsular Malaysia and their implications for conservation. In: Sahir O, Siti Hawa Y, Sivanathan E, Shukor MN, Norhayati A, Shahrul AMS (Eds) Management and Status of Resources in Protected Areas of Peninsular Malaysia. Department of Wildlife and National Parks Kuala Lumpur, Malaysia, 175-198.

Kawanishi K, Gumal M, Shepherd LA, Goldthorpe G, Shepherd CR, Krishnasamy K, Hashim AKA (2010) The Malayan Tiger. In: Tilson R, Nyhus PJ (Eds) Tiger of the World (2nd edn.). Elsevier Inc., 376-376. https://doi.org/10.1016/B978-0-8155-1570-8.00029-3

Khairulmunir M, Gani M, Karuppannan KV, Mohd-Ridwan AR, Md-Zain BM (2023) High-throughput DNA metabarcoding for determining the gut microbiome of captive critically endangered Malayan tiger (*Panthera tigris jacksoni*) during fasting. Biodiversity Data Journal 11: e104757. https://doi.org/10.3897/BDJ.11.e104757

Kim BR, Shin J, Guevarra R, Lee JH, Kim DW, Kuk-Hwan S, Ju-Hoon L, Kim HB, Isaacson RE (2017) Deciphering diversity indices for a better understanding of microbial communities. Journal of Microbiology and Biotechnology 27(12): 2089–2093. https://doi.org/10.4014/jmb.1709.09027

Kolipaka SS, Tamis WLM, van 't Zelfde M, Persoon GA, de Iongh HH (2017) Wild versus domestic prey in the diet of reintroduced tigers (*Panthera tigris*) in the livestock-dominated multiple-use forests of Panna Tiger Reserve, India. PLoS ONE 12(4): e0174844. https://doi.org/10.1371/journal.pone.0174844

Kumar S (2015) Prey selection and food habit of tiger (*Panthera tigris*) in Corbett Tiger Reserve, Ramnagar, Uttrakhand, India. International Journal of Innovative Research and Development 4(8): 212–215.

Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. Frontiers in Zoology 10(1): 34. https://doi.org/10.1186/1742-9994-10-34

Lu Q, Xiao L, Cheng C, Lu Z, Zhao J, Yao M (2021) Snow leopard dietary preferences and livestock predation revealed by fecal DNA metabarcoding: No evidence for apparent competition between wild and domestic prey. Frontiers in Ecology and Evolution 9: 783546. https://doi.org/10.3389/fevo.2021.783546

Lynam AJ, Laidlaw R, Noordin WSW, Elagupillay S, Bennet EL (2007) Assessing the conservation status of the tiger *Panthera tigris* at priority sites in Peninsular Malaysia. Oryx 41(4): 454–462. https://doi.org/10.1017/S0030605307001019

Mansor MS, Rozali FZ, Davies S, Nor SM, Ramli R (2022) High-throughput sequencing reveals dietary segregation in Malaysian babblers. Current Zoology 68(4): 381–389. https://doi.org/10.1093/cz/zoab074

Matthews JK, Ridley A, Kaplin BA, Grueter CC (2020) A comparison of fecal sampling and direct feeding observation for qunatifying the diet frugivorous primate. Current Zoology 66(4): 333–343. https://doi.org/10.1093/cz/zoz058

McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8(4): e61217. https://doi.org/10.1371/journal.pone.0061217

Mohd-Radzi NHS, Karuppannan KV, Abdullah-Fauzi NAF, Mohd-Ridwan AR, Othman N, Abdul-Latiff MAB, Gani M, Abdul-Razak MFA, Md-Zain BM (2022) Determining the diet of wild Asian elephants (*Elephas maximus*) at human-elephant conflict areas in Peninsular Malaysia using DNA metabarcoding. Biodiversity Data Journal 10: e89752. https://doi.org/10.3897/BDJ.10.e89752

Monterroso P, Godinho R, Oliveira T, Ferreras P, Kelly MJ, Morin DJ, Waits LP, Alves PC, Mills LS (2018) Feeding ecological knowledge: The underutilised power of faecal DNA approaches for carnivore diet analysis. Mammal Review 49(2): 97–112. https://doi.org/10.1111/mam.12144

Mukherjee S, Sarkar NS (2013) The range of prey size of the Royal Bengal Tiger of Sundarbans. Journal of Ecosystems 351756: 1–7. https://doi.org/10.1155/2013/351756

Mumma MA, Adams JR, Zieminski C, Fuller TK, Mahoney SP, Waits LP (2015) A comparison of morphological and molecular diet analyses of predator scats. Journal of Mammalogy 97(1): 112–120. https://doi.org/10.1093/jmammal/gyv160

Nielsen JM, Clare EL, Hayden B, Brett MT, Kratina P (2018) Diet tracing in ecology: Method comparison and selection. Methods in Ecology and Evolution 9(2): 278–291. https://doi.org/10.1111/2041-210X.12869

Osman NA, Abdul-Latiff MAB, Mohd-Ridwan AR, Yaakop S, Nor SM, Md-Zain BM (2020) Diet Composition of the Wild Stump-Tailed Macaque (*Macaca arctoides*) in Perlis State Park, Peninsular Malaysia, Using a Chloroplast tRNL DNA Metabarcoding Approach: A Preliminary Study. Animals 10(12): 2215. https://doi.org/10.3390/ani10122215

Osman NA, Abdul-Latiff MAB, Mohd-Ridwan AR, Yaakop S, Karuppannan KV, Md-Zain BM (2022) Metabarcoding data analysis revealed the plant dietary variation of long-tailed macaque *Macaca fascicularis* (Cercopithecidae, Cercopithecinae) living in disturbed habitats in Peninsular Malaysia. Biodiversity Data Journal 10: e89617. https://doi.org/10.3897/BDJ.10.e89617

Pounsin G, Wahab NS, Roslan A, Zahidin MA, Pesiu E, Tamrin NAM, Abdullah MT (2018) Diversity of bats in contrasting habitats of Hulu Terengganu Dipterocarp Forest and Setiu Wetland BRIS Forest with a note on preliminary study of vertical stratification of Pteropodid Bats. Tropical Life Sciences Research 29(1): 51–69. https://doi.org/10.21315/tlsr2018.29.1.4

Quéméré E, Aucourd M, Troispoux V, Brosse S, Murienne J, Covain R, Pierre-Yves LB, Olivier J, Tysklind N, Galan M (2021) Unraveling the dietary diversity of Neotropical top predators using scat DNA metabarcoding: A case study on the elusive Giant Otter. Environmental DNA 3(5): 889–900. https://doi.org/10.1002/edn3.195

Ramesh T, Snehalatha V, Sankar K, Qureshi Q (2009) Food habits and prey selection of tiger and leopard in Mudumalai Tiger Reserve, tamil Nadu, India. Scientific Transactions in Environment and Technovation 2(3): 170–181. https://doi.org/10.20894/STET.116.002.003.010

Reddy HS, Srinivasulu C, Rao KT (2004) Prey selection by the Indian tiger (*Panthera tigris tigris*) in Nagarjunasagar Srisailam Tiger Reserve, India. Mammalian Biology 69(6): 384–391. https://doi.org/10.1078/1616-5047-00160

Ritchie EG, Elmhagen B, Glen AS, Letnic M, Ludwig G, McDonald RA (2012) Ecosystem restoration with teeth: What role for predators? Trends in Ecology & Evolution 27(5): 265–271. https://doi.org/10.1016/j.tree.2012.01.001

Rytkönen S, Vesterinen EJ, Westerduin C, Leviäkangas T, Vatka E, Mutanen M, Välimäki P, Hukkanen M, Suokas M, Orell M (2018) From feces to data: A metabarcoding method for analyzing consumed and available prey in a bird-insect food web. Ecology and Evolution 9(1): 631–639. https://doi.org/10.1002/ece3.4787

Sato JJ, Ohtsuki Y, Nishiura N, Mouri K (2022) DNA metabarcoding dietary analyses of the wood mouse *Apodemus speciosus* on Innoshima Island, Japan, and implications for primer choice. Mammal Research 67(1): 109–122. https://doi.org/10.1007/s13364-021-00601-7

Schaller GB (1967) The deer and the tigers: a study of wildlife in India. University of Chicago Press, Chicago, USA.

Shevade VS, Potapov PV, Harris NL, Loboda TV (2017) Expansion of industrial plantations continues to threaten Malayan tiger habitat. Remote Sensing 9(7): 747. https://doi.org/10.3390/rs9070747

Simcharoen A, Savini T, Gale GA, Simcharoen S, Duangchantrasiri S, Pakpien S, Smith JL (2014) Female tiger Panthera tigris home range size and prey abundance: Important metrics for management. Oryx 48(3): 370–377. https://doi.org/10.1017/S0030605312001408

Srivathsan A, Ang A, Vogler AP, Meier R (2016) Fecal metagenomics for the simultaneous assessment of diet, parasites, and population genetics of an understudied primate. Frontiers in Zoology 13(1): 17. https://doi.org/10.1186/s12983-016-0150-4

Sunquist ME, Sunquist FC (1989) Ecological constraints on predation by large felids. Carnivore Behavior, Ecology, and Evolution: 283–301. https://doi:org/10.1007/978-1-4757-4716-4_11

Ten DCY, Jani R, Hashim NS, Saaban S, Abu-Hashim AK, Abdullah MT (2021) *Panthera tigris jacksoni* population crash and impending extinction due to environmental perturbation and human-wildlife conflict. Animals 2021(11): 1032. https://doi.org/10.3390/ani11041032

Tercel MPTG, Moorhouse-Gann RJ, Cuff JP, Drake LE, Cole NC, Goder M, Mootoocurpen R, Symondson WOC (2021) DNA metabarcoding reveals introduced species predominate in the diet of a threatened endemic omnivore, Telfair's skink (*Leiolopisma telfairii*). Ecology and Evolution 12(1): e8484. https://doi.org/10.1002/ece3.8484

Thuo D, Furlan E, Broekhuis F, Kamau J, Macdonald K, Gleeson DM (2019) Food from faeces: Evaluating the efficacy scat DNA metabarcoding in dietary analyses. PLoS One 14(12): e0225805. https://doi.org/10.1371/journal.pone.0225805

Topani R (1990) Status and distribution of tiger in Peninsular Malaysia. Journal of Wildlife and Parks 9: 71–102.

Traugott M, Thalinger B, Wallinger C, Sint D (2020) Fish as predators and prey: DNA-based assessment of their role in food webs. Journal of Fish Biology 98(2): 367–382. https://doi.org/10.1111/jfb.14400

Upadhyaya SK, Musters CJM, Lamichhane BR, de Snoo GR, Thapa P, Dhakal M, Karmacharya D, Shrestha PM, de Longh HH (2018) An insight into the diet and prey preference of tigers in Bardia National Park, Nepal. Tropical Conservation Science 11: 1–9. https://doi.org/10.1177/1940082918799476

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://doi.org/10.1007/978-3-319-24277-4

Xiong M, Wang D, Bu H, Shao X, Zhang D, Li S, Wang R, Yao M (2017) Molecular dietary analysis of two sympatric felids in the mountains of Southwest China biodiversity hotspot and conservation implications. Scientific Reports 7(1): 41909. https://doi.org/10.1038/srep41909